Infections in surgical patients remain a significant cause of morbidity and mortality despite advances made in source control, antimicrobial therapy, and adjunctive therapies. Although there is much focus on optimizing the use of currently available therapy and the translation of evidence into practice,1–5 there are a wide variety of innovations that might prove to be critical assets in the battle between microbes and their human host.

These innovations come at a critical time. The ability to support acute organ failure through advances in critical care or chronic organ failure through transplantation and immunosuppression is being challenged by the threat of increasing antimicrobial resistance. As a result, many have emphasized the urgent need for novel strategies either to prevent or treat infections caused by multidrug-resistant organisms.6–10

A number of diagnostic and therapeutic modalities currently in experimental phases of development have the potential not only to improve patient outcomes, but also to provide novel approaches to bypassing microbial resistance mechanisms, and represent potentially sustainable strategies in slowing the current rate of antimicrobial resistance. These innovative approaches include new and rapid diagnostic tests that could limit exposure to broad-spectrum antimicrobials through quicker diagnosis and more timely de-escalation; novel microbial targets to reduce pathogenicity; vaccine approaches to infection prevention; and prognostic markers to identify those at highest risk.
Antibiotic exposure is the principal driving force behind emerging resistance. Many antimicrobial guidelines emphasize the need to avoid the use of antimicrobials in clinical settings where they are unlikely to be of benefit, to narrow the spectrum when possible, and to reduce the duration of exposure. These goals are counter to those that might be in the best interest of the patient, as early empiric broad-spectrum therapy has been associated with a survival benefit.

The current approach to antimicrobial prescribing is in conflict with what might be ideal practice because of currently used microbial diagnostics. Conventional culture and microbial identification techniques typically require almost 72 hours for pathogens to be identified, speciated, and susceptibility patterns established. During this time of diagnostic uncertainty, the patient, particularly the critically ill patient, is treated with broad-spectrum antibiotics, with de-escalation of therapy delayed until a definitive diagnosis is made. Moreover, conventional cultures may not identify the offending pathogen. This is particularly true of fastidious or slow-growing organisms, which include not only unusual pathogens, but also many fungal pathogens and a number of commonly encountered bacteria. In addition, cultures taken after the initiation of antimicrobial therapy may have low yield, despite the presence of clinically important infection.

The need to balance efforts to minimize the use of antimicrobials and to treat the patient adequately during a period of diagnostic uncertainty has led to the development of novel diagnostic strategies for the detection of pathogenic organisms based on advances in genomics and molecular biology.

**Rapid Diagnostic Assessment**

Traditionally, patient specimens are collected and then cultured in automated systems that detect the presence of any bacterial or fungal growth. If a culture “flags” positive, the pathogen is examined by means of Gram staining, which offers some diagnostic clues as to the nature of the microorganism, and is then further cultured and examined by various biochemical assays. Antibiotic susceptibilities are also examined during this period. New diagnostic assays use a variety of molecular diagnostic techniques to accelerate the diagnosis. The most common techniques include fluorescent in situ hybridization (FISH), polymerase chain reaction (PCR) amplification of unique genetic sequences, and microarray-based technology.

FISH relies on the use of a suspension of molecular probes that bind to a marker specific to a particular pathogen (eg, a highly specific ribosomal RNA). The probes have a detection system comprised of fluorescent markers that can be visualized by microscopy. PCR relies on the exponential amplification of microbial genetic material through an automated system; only a particular segment of genetic information is targeted by the assay, which ensures that if no pathogen with the genetic sequence is present in the sample, no amplification occurs. The amplified product is then detected, either directly or indirectly, through the presence of an automated detection system. Finally, microarray technology refers to the practice of fixing a large number of highly specific probes onto a stable medium, thereby enabling analysis of a large number of DNA or RNA sequences in an automated fashion. Each of these techniques has a large number of variations, and they are frequently used in tandem.

**Culture-Based Assays**

The majority of newer diagnostic assays use specimens derived from microbial cultures after specimen collection, as opposed to immediae analysis of specimens.
obtained from the patient. Identifying pathogens from culture-positive specimens has a number of potential advantages. It ensures that only cultures with live bacteria result in a positive test. It also ensures that the appropriate molecular probes are used through preliminary information obtained through Gram staining and early culture. An excess number of probes in a single reaction can lead to a greater chance of a false-positive assay, and the information obtained through Gram staining and culture can help tailor the choice of probes, limit costs, and increase the chance of a valid result.

A wide range of assays aiming to identify pathogens from positive blood cultures have been designed. Although some are designed to detect only one or two specific pathogens, many have been developed to assess for the presence of a wide spectrum of organisms. As an example of the diagnostic utility of some of these assays, Oliveira and coworkers developed a FISH assay to detect *Staphylococcus aureus* from a positive blood culture specimen. Results diverged from conventional culture results in less than 1% of cases, and results were available within 2.5 hours of a positive culture. Marlowe and coworkers described a DNA probe matrix designed to identify a variety of gram-positive, gram-negative, and fungal pathogens within 1 hour of positive culture. The assay demonstrated less than 2% discordance with conventional cultures.

There is considerable variability, however, in accuracy across the reported studies. For example, a FISH assay designed to detect a wide range of pathogens identified species in only 81% of cases with positive conventional cultures. This loss of sensitivity was caused by an inadequate breadth of gram-negative probes, illustrating some of the inherent limitations of the high specificity of molecular diagnostic techniques. Specifically, there must be a probe present for all potential organisms. Other assays designed to detect a wide range of pathogens, by FISH or amplification methods, have reported similar difficulties in identifying all clinically encountered pathogens to the species level. These assays are not quite ready for widespread use in the clinical setting.

**Other Uses for Molecular Diagnostic Strategies**

There are several other potential applications for molecular diagnostic strategies that might prove valuable in the future. In part, these applications provide greater hope for success because they focus on particular organisms or clinical problems rather than casting a wide diagnostic net. For example, molecular diagnostic assays have proved to be useful in the rapid detection of antibiotic resistance genes. Several assays have been developed that can detect the presence of multiple genes concurrently in a matter of hours. One study examined an oligonucleotide microarray that could, through PCR amplification, detect 90 different antibiotic resistance genes in gram-positive bacteria. Another assay used microarray analysis to detect 61 different resistance genes from different species of bacteria, and to identify instances of horizontal gene transfer. Nevertheless, the practical limitation of this approach is the constant evolution of new resistance genes, or their transfer to new pathogens, which requires a continuous updating of available assays.

Where the molecular diagnostic approach does seem to offer an advantage is in the rapid detection of methicillin-resistant *S aureus* (MRSA), a phenotype generally conferred by the mecA gene in *S aureus*. *S aureus* is the most common blood-borne pathogen and the most important pathogen in the postoperative period. Further, the increasing prevalence of MRSA has altered the approach to both prevention and treatment of suspected infections caused by *S aureus*. New diagnostic assays might simplify the approach to the identification and treatment of staphylococcal...
infections. For example, the preliminary Gram stain of a blood culture result might be “gram-positive cocci in clusters.” Most commonly, this result might represent a clinically insignificant contaminant with coagulase-negative staphylococci, or a bacteremia caused by methicillin-sensitive \textit{S. aureus} or MRSA. Several groups have developed PCR and microarray-based assays that discriminate between \textit{S. aureus} and coagulase-negative staphylococci with 100% reliability within hours of a positive culture.\textsuperscript{38–41} More importantly, although genomic analysis does not always correlate precisely with phenotypic resistance patterns, multiple groups have described PCR-based assays with an accuracy of greater than 95% for the detection of MRSA in mixed cultures.\textsuperscript{39,42–44}

The clinical utility of rapid diagnosis of \textit{S. aureus} bacteremia and identification of MRSA has been demonstrated in a number of studies. In a retrospective study where PCR was performed on specimens derived from patients with a positive conventional blood culture, the PCR result, if available, would have better directed therapy in a significant proportion of patients through a significant reduction in the use of vancomycin.\textsuperscript{39} In other studies comparing conventional and molecular diagnostic methods, rapid results regarding MRSA status would have changed therapy in over 25% of patients.\textsuperscript{42,45} PCR detection of MRSA directly from patients’ specimens has not only been shown to decrease MRSA infection rates, but has been shown to be cost-effective in the clinical setting.\textsuperscript{46}

Like MRSA detection, postoperative fungemia represents another clinical domain in which new diagnostic techniques could prove to be invaluable. Fungal organisms are frequently difficult to detect and are associated with significant morbidity and mortality. Further, it might take as long as 4 to 5 days to reliably declare a blood culture negative for fungal pathogens. Novel diagnostic strategies using PCR or FISH have shown tremendous promise in the rapid detection of these pathogens.\textsuperscript{47–55} For example, Lau and coworkers\textsuperscript{56} reported a PCR-based assay capable of detecting 11 species of fungus from positive blood cultures. This assay correctly identified all cultures that contained pathogens targeted by the assay with great accuracy in as little as 4 hours. Another assay using FISH to detect \textit{Candida albicans} from positive blood cultures even demonstrated improved sensitivity compared with conventional methods.\textsuperscript{57}

These fungal assays vary considerably in their complexity and costs, but many are likely to be cost-effective given the changing face of fungal infections. The widespread use of fluconazole has been associated with the emergence of resistant fungi including non-albicans \textit{Candida} and non-fumigatus \textit{Aspergillus}.\textsuperscript{8} As a result, caspofungin or other more costly antifungals might be required as empiric therapy in certain clinical settings.\textsuperscript{58} Rapid detection systems for fungal pathogens might allow for more rapid de-escalation and overall cost savings.\textsuperscript{59,60}

\textbf{Specimen-Based Assays}

The primary disadvantage of the previously described molecular diagnostic techniques is their reliance on a period of in vitro culture of the organism. With the advent of newer diagnostic techniques that might directly detect pathogens in specimens without the need for prior culture, there is the potential for further reducing the time to diagnosis. PCR-based assays designed to detect \textit{S. aureus} and \textit{Enterococcus faecalis} directly from blood samples have been developed.\textsuperscript{61} Of greater use might be a recently described multiplex PCR assay that detects the presence of multiple gram-negative, gram-positive, and fungal organisms in blood samples, and which provides results within 7 hours of specimen collection.\textsuperscript{62,63} Most specimen-based assays have sensitivities in the range of 65% to 75%, however, particularly with clinical specimens, too low to be of clinical use.\textsuperscript{61–64} This lower sensitivity is likely caused by
the presence of other compounds that interfere with the sensitive reactions on which these diagnostic tests rely. Although these assays hold promise, there are significant technical limitations that need to be overcome.

Pathogen Profiling

In addition to providing conventional information faster, molecular diagnostics are improving to the point that they will soon provide a new class of diagnostic information. Specifically, it will likely be possible to generate a more comprehensive, genetically based profile of the organism to better estimate the potential for pathogenicity or contribution to the clinical picture. This pathogen profiling provides information regarding the organism of interest, including the presence of resistance genes and virulence factors, and acknowledges the tremendous within-species and temporal variability in gene expression. With the data accruing over time, this information could be linked to the probability of a clinically relevant phenotypic behavior and could be used to guide clinical decision-making. This approach would provide not only information about the pathogen’s present phenotype (potentially in a more timely manner), but also might provide insight into the likely future behavior of the pathogen in vivo. For example, pathogen profiling has been used to guide use, dose, and duration of antiviral therapy in the context of hepatitis C infection. Pathogen profiling has also been applied to the evaluation of a wide range of pathogens, including Escherichia coli, Pseudomonas aeruginosa, and S aureus strains, and to their virulence factors. With further experience it might soon be possible to determine whether an organism is a colonizer or a pathogen and its propensity for the development of antimicrobial resistance. The rapid transmission of this information to the clinician might have direct impact on the approach to any identified organism.

NOVEL APPROACHES TO THERAPEUTICS

The discovery of effective and relatively nontoxic antimicrobial agents from environmental sources has historically led to a focus on natural sources of new antibacterial and antifungal agents. Few antimicrobials are purely synthetic in origin, and potential antimicrobials are typically identified by screening candidate compounds against live pathogens. Compounds identified by this method target molecular processes required by the organism for survival or for propagation. By definition, this approach has led to antimicrobials that stimulate bacterial evolution by making high mutation rates more adaptive, and leads to resistance.

A proposed alternative is the targeting of so-called “virulence factors,” bacterial products that play a causal role in the manifestations of disease either by enabling the steps that lead to infection, or by causing disease symptoms (eg, secreted toxins), but which are not essential to the pathogen’s survival. It is thought that such antimicrobials, which may cause less selection pressure, would be less likely to lead to the rapid emergence of resistance.

Although antimicrobial strategies targeted at virulence factors are as varied as the potential mechanisms of virulence, the greatest future success might lie in targeting bacterial quorum-sensing pathways. Quorum-sensing, which is the mechanism by which bacterial populations modify their gene expression in response to changes in the density of surrounding organisms, is closely linked to pathogenic behavior in many bacterial species. This behavior includes host invasion; surface adhesion; intercellular signaling; evasion of host defenses; and modification of the host environment (eg, toxins). This strategy holds tremendous promise for therapy and might also
play an important role in limiting the surface adhesion and biofilm formation that is a necessary first step in infections of surgical implants.

Quorum-sensing seems to be so critical to bacterial pathogenicity that therapies against this target may be highly effective. For example, inhibition of the quorum-sensing cascade in a murine model of *S aureus* infection blocked abscess formation. Further, this effect was evident with only transient blockade of the quorum-sensing cascade in the earliest phases of infection.\textsuperscript{73} RNAIII-inhibiting peptide (RIP), which inhibits quorum-sensing in *S aureus* without decreasing bacterial cell counts, has been found to be potentially useful in a number of models. When administered as an antibiotic lock therapy to animals in a model of catheter-related bacteremia, catheter colonization was reduced after injection of *S aureus* directly into the catheter, and completely eliminated when RIP was used in combination with antibiotics.\textsuperscript{74} In a murine model of polyethylene terephthalate (Dacron) graft infection, presoaking the graft with RIP decreased the growth of *S aureus* by a thousand-fold.\textsuperscript{75,76} Further, a compound that is a nonpeptide analogue of RIP, and occurs naturally in the bark of witch hazel, interfered with bacterial adhesion and biofilm formation in an in vitro model, and demonstrated a dose-dependent inhibition of graft colonization in a murine model.\textsuperscript{77} Finally, quorum-sensing has been targeted through the generation of monoclonal antibodies against AIP-4, which is involved in the quorum-sensing system of *S aureus*. Passive immunization with these antibodies resulted in increased survival in a murine model of *S aureus* intraperitoneal sepsis.\textsuperscript{78}

Quorum-sensing mechanisms have also been well documented and extensively studied in another common hospital-acquired pathogen, *P aeruginosa*. It has been demonstrated, for example, that azithromycin interferes in quorum-sensing pathways of *P aeruginosa*, and may be potentially beneficial in patient populations susceptible to chronic infections by this pathogen.\textsuperscript{79} The therapeutic potential of targeting quorum-sensing is also evident in another experimental approach, involving immunization against a pseudomonal autoinducer. This increased survival in a murine model of *P aeruginosa* pulmonary infection, but resulted in no change in bacterial cell counts.\textsuperscript{80} This suggests that the observed decrease in mortality was caused by prevention of bacterial pathogenicity, rather than by growth inhibition. Targeting quorum-sensing pathways is not universally beneficial, however, and there seems to be dose-related effects that might even be harmful. The complex nature of quorum-sensing has been highlighted in this species in experiments demonstrating that subtherapeutic doses of other common agents, such as tetracycline, tobramycin, and ciprofloxacin, paradoxically induced genes associated with biofilm formation, and that subtherapeutic doses of tobramycin promote motility.\textsuperscript{81,82}

Based on evidence to date, therapies targeted at bacterial virulence factors potentially offer a highly attractive combination: decreased pathogenicity without interference in cell replication, and a decreased likelihood of creating a strong selective pressure for resistance. Although the described therapies have not been evaluated in clinical trials, therapies aimed at virulence factors may be highly promising novel antimicrobials.

### INFECTION PREVENTION THROUGH VACCINATION

Vaccines have likely been, historically, the most effective medical intervention for the prevention of infection. The advent of pathogen profiling and a broader understanding of the relationship between organism and host have led to a more sophisticated approach to vaccine development. Many of the pathogens commonly seen in surgical practice, such as *S aureus* and group A streptococci (GAS), have not been, until
recently, preventable through vaccine strategies. A major obstacle in the development of effective vaccines against these pathogens is the high degree of variability of their surface antigens. The large number of resultant serotypes, the number of which is further increased by bacterial phase variation, makes creating a vaccine that results in universal resistance against the pathogen challenging.83,84

There are efforts underway to develop vaccines effective in preventing infections caused by *S aureus* given the burden of disease caused by this organism. Animal experiments and clinical trials have met with mixed results. A vaccine based on the *S aureus* capsular polysaccharide types 5 and 8, which account for 93% of clinical isolates, was evaluated in a large cohort of patients with end-stage renal disease.85,86 The vaccine did not reduce the incidence of *S aureus* bacteremia over the year of observation; however, additional analyses suggest that periodic boosters might improve the vaccine’s efficacy.

In a more recent study, animals were vaccinated with a nontoxic peptide similar in structure to alpha hemolysin, a cytotoxin produced by *S aureus*, which resulted in the production of an antibody directed against the active peptide. This antibody proved protective in a murine model of *S aureus* pneumonia.87 *S aureus* quorum-sensing has also been used as a vaccine target. For example, immunization against a bacterial product involved in staphylococcal quorum-sensing resulted in increased survival in a murine model of *S aureus* pneumonia.88

*GAS* is a highly virulent organism, and like *S aureus* is of considerable surgical importance given its role in severe skin and soft tissue infections. As a result, many investigators have focused on developing effective vaccines directed against this organism or its virulence factors. The M protein of GAS, of which there are at least 100 variants, is responsible for specific immunity against this pathogen. In addition, antibodies directed against the M protein cross-react with certain human antigens. Taken together, these two factors significantly complicate the development of a safe and effective vaccine.89,90 Recent analyses, however, have identified regions of the M protein that are both highly antigenic and specific to GAS. This has resulted in the construction of a polyvalent vaccine that has undergone phase I studies in humans.90 It is estimated the vaccine could prevent up to 50% cases of invasive disease in adults, and an equal proportion of GAS-related deaths.89

Some of the challenges associated with identifying a universal vaccine target for species with highly variable antigens have recently been simplified with novel genomics-based approaches. “Reverse vaccinology” refers to the process of screening genomic sequences for regions that are likely to represent potential vaccine targets, such as cell membrane proteins that are conserved across species subtypes.83 This approach has been used to identify potential vaccine targets for *Streptococcus pneumoniae*,91 GAS,92 and *Neisseria meningitidis*.93,94 This approach has also been used to create a potential *S aureus* vaccine, which in preliminary studies has been demonstrated to provide protective immunity against *S aureus* from human clinical isolates in a murine model.95

Another innovative methodology in vaccine development is the use of the so-called “antigenome” approach to vaccine target identification.96 This approach uses antibodies collected from convalescent patients to screen antigen expression libraries. In this way, only genes that produce antigens to which patients produce antibodies are selected for further study. This limits the number of targets studied, and identifies targets that are actually expressed in vivo. The antigenome strategy has, for example, been successfully used in *S aureus* vaccine development where it has been proved efficacious in murine models.97 More importantly, this vaccine generated a strong immunologic response in primates despite pre-existing antibodies, suggesting that
the vaccine could be used as a nosocomial vaccine. Similar approaches have been used by other groups for *S. aureus* and for *S. pneumoniae*.96,98,99

Infection control through vaccination holds great promise. Although the described therapies require many years of evaluation, a vaccine that might confer a moderate degree of protection could have significant effects on the burden of disease without the selection pressures associated with antimicrobial therapy. The challenge will then be to target those at highest risk for infections, a daunting task given our relatively primitive understanding of host-microbial interactions.

ADVANCES IN UNDERSTANDING HOST-MICROBIAL INTERACTIONS

The wide variation in outcomes following infection emphasizes the multiple factors that play a role in dictating the host’s response to a microbial challenge. The host’s response to infection might very well play a greater role in outcome than either the clinical context or the implicated pathogen. The observation that individuals differ in their susceptibility to infection over and above the known clinical risk factors has been well documented.100 Recent advances in high throughput genomics may allow identification of high-risk individuals by virtue of their genotypes. Specifically, several single nucleotide polymorphisms, which represent minor genetic variations that occur in a small proportion of the population, have been associated with adverse outcomes related to infection.100,101

A systematic review of genes investigated for polymorphisms linked to the response to infection identified 76 studies examining 51 different genetic polymorphisms.102 Genes investigated to date relate to those previously implicated in the host response to infection, including genes associated with cytokine production, the coagulation cascade, and signal transduction.103,104 As the associated technology becomes increasingly rapid and affordable, however, it will likely become possible to scan the genome for genes associated with susceptibility to infectious disease without prior knowledge of their function.100

Polymorphisms related to tumor necrosis factor (TNF)-α and the Toll-like receptor (TLR) family have been among the more extensively studied given the current understanding of the role of these proteins in the manifestations of infection. TNF-α is a proinflammatory mediator that is produced in response to a wide range of stimuli, and that has been implicated in a number of inflammatory diseases, including sepsis.101 The prevalence of the TNF2 polymorphism has been found to be significantly higher among patients with septic shock than the general population,105,106 and has been found to be an independent predictor of death among patients with septic shock.105 Among surgical patients, TNF-α polymorphisms have been associated with adverse outcomes among patients with postoperative septic shock.107

TNF-α polymorphisms have also been associated with surgical site or other nosocomial infections, suggesting it might be possible to identify those predisposed to infection and to better target preventive measures. In a study of patients undergoing esophagectomy, there was an association between TNF-α polymorphisms and subsequent infection.108 The relationships are not simple, however, as the polymorphisms associated with infection in this study were found to confer protection in others.105,106 Polymorphisms of the TNF-α gene have also been associated with increased susceptibility to sepsis following severe burns and trauma.109–111

Similar associations have been identified when polymorphisms pertaining to the TLR receptor family have been evaluated. These receptors are key in recognizing bacterial cell wall components and initiating the inflammatory cascade.112 The TLR4 mutation has been associated with increased susceptibility to gram-negative infection
among ICU patients\textsuperscript{113} and in patients with major burns.\textsuperscript{109} Others have demonstrated an association between a polymorphism at the TLR2 gene and increased susceptibility to gram-positive and fungal infection among patients with septic shock.\textsuperscript{114,115}

Although this line of investigation might yield promise in identifying those at highest risk for infection, and those who might benefit from novel interventions, many of these results are inconsistent across studies.\textsuperscript{108,116–122} The inconsistent findings might be caused by differences in methodology, small sample size, confounding through cosegregation of genetic loci, or gene interactions that are only poorly understood.\textsuperscript{100,102,112}

It is anticipated that over time these investigations will yield considerable insights into risk factors (and prognosis) related to infection. These association studies must be performed using large populations of patients representing diverse ethnic and racial backgrounds, however, to ensure the findings are generalizable to the population at large, and they must consider the complex interactions between genetic polymorphisms at multiple gene loci.\textsuperscript{103,123,124} If these methodologic issues can be overcome, the clinical benefits would be tremendous. Additionally, these studies would then allow for evaluation of new pathways that play a role in the development or manifestations of infection, and provide new avenues of study for targeted interventions. These novel interventions would be directed against the host response or host-microbial interactions, and would not be encumbered by the adverse selection pressures associated with antimicrobial therapy.

\textbf{SUMMARY}

Innovations in the field of infection hold significant promise in the way surgical infections are prevented, diagnosed, and treated. Diagnostics will allow for more rapid assessment of the causal pathogen and the likelihood of any particular organism contributing to the manifestations of disease. Treatment strategies will focus not on adding further selection pressures, but on compromising microbial virulence factors to render them innocuous. We are still in the early phases of experimenting with vaccine strategies for infection prevention in this clinical setting, but newer techniques might allow the development of effective vaccines for common nosocomial pathogens. Finally, the ability to identify patients at highest risk for infection through analyses of genetic polymorphism provides an opportunity to better understand host-microbial interactions and to target novel interventions.

\textbf{REFERENCES}


